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Constituent Acids of *Cucumis sativus* Fruit Cutin

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The cutin from fruit peel of *Cucumis sativus* was found to constitute n-alkanoic (C_{12}—C_{36}), α, ω-alkandioic (C_9,C_{16},C_{18} and C_{22}) hydroxy-alkanoic (C_{14}—C_{23}), hydroxy α, ω-alkandioic (C_{12}—C_{32}) and benzoic acid together with hippuric acid. The main constituents were octadecandioic acids (*ca.* 13%), 8,10-dihydroxy octadecandioic acid (*ca.* 11%) and octadecendioic acid (*ca.* 10%).

INTRODUCTION

The plant cuticle is believed to be the primary barrier to penetration of foliar applied chemicals and also a protective barrier against ingress of pathogens[1–5]. Movement of such chemicals through the cuticle may occur by diffusion whereas fungal penetration may involve enzymatic hydrolysis of the cuticle by weakening the barrier. Retention is an important prerequisite for penetration, before a biological response can be induced. The structural component of plant cuticle, cutin, is an insoluble bio-polyester composed of interesterfied hydroxy and hydroxy-epoxy fatty acids embedded in a complex mixture of relatively nonpolar lipids called waxes. The surface fine-structure and chemical composition of epicuticular waxes and the chemical composition or deposition of cutin may vary within different anatomical region of the plant as well as alter significantly due to pesticide effects on plant cuticle.

The examination of the chemical composition of cutin from fruit peel of *Cucumis sativus* was undertaken as a part[6] of a general investigation of the physical and chemical properties of surface layer. The *C. sativus* fruit has not been previously studied in detail for its cutin compositional analysis except some kinetic studies[7] relating to sorption of 2,4,-D by the cuticle.

In this paper we report the compositional studies and structural characterisation of monomers from *C. sativus* fruit peel by GC-EI-MS.

EXPERIMENTAL

Fresh mature fruits of *Cucumis sativus* were collected from local market in July. Segments of fruits peel (1 cm × 1 cm) size were immersed successively in $CHCl_3$ and MeOH (2 h in each solvent), dried in air. Cellulose was removed by immersing in a mixture of $ZnCl_2$ and conc. HCl (1 : 2) for 24 h. These were washed with distilled water and repeated treatment with $ZnCl_2$—HCl was done. After thorough washing, the cuticles were extracted in a soxhlet with MeOH and then pectin was removed by refluxing with a solution of 1.6% ammonium oxalate and 0.4% oxalic acid. The membranes were once again washed, air dried and extracted with $CHCl_3$—MeOH (1 : 1) using soxhlet to remove residual lipids. The cutin thus obtained was dried *in vacuo* and powdered (presence of ester group was confirmed from IR spectra).

Dry cutin (50 mg) was refluxed with 100 mL of 5% KOH in MeOH for 3 h to achieve depolymerization. After removal of MeOH the resulting solution was acidified with conc. HCl and extracted with Et_2O. After removal of Et_2O, resulting material was dried over P_2O_5.

The cutin acids thus produced was methylated with CH_2N_2. The methylated esters were injected directly in to the GC and GC—MS systems. GC was performed on 25 M SP-1000 and SP-2100 fused silica SCOT capillary column, programmed from 80° (5 min) to 220°C at the rate of 4°C/min (maintained isothermally for 67 min) and from 210°C (6 min) to 330°C at the rate of 6°C/min (maintained isothermally for 4 min) respectively. Helium was used as carrier gas (1.0 mL/min) in either condition. GC-MS was performed at 72 ev with a split (1:60) injection system and a computerized library search facility.

RESULTS AND DISCUSSION

The prepared cutins were hydrolyzed with 5% KOH in MeOH and the reaction mixture worked up as follows: removal of MeOH, acidification with aqueous HCl, extraction of ether soluble components and methylation using diazomethane. The methyl ester was then analysed by GC and GC-EI-MS. The identification of each component was made either through direct comparison of mass spectral data using computerized library searching or by analysing the mass spectral fragementation patterns. The highly preferred α-cleavage on either side of the midchain —OH substituent makes the identification by their mass spectra quite convenient. The composition of cutin from a sample of *C. sativus* fruit peel is summarized in Table-1. Although conversion of cutin acids to the methyl ester TMSi ethers

TABLE-1
COMPOSITION OF *C. SATIVUS* FRUIT CUTIN MONOMER ACIDS AS DETERINED BY HYDROLYSIS

Monomers	Relative percentages
Alkanoic acids (C_{12}—C_{18})	23.5
Alkanoic acids (C_{20}—C_{36})	2.6
Alkandioic acids	~30.0
ω-Hydroxyalkanoic acids	4.6
Midchain hydroxylated acids:	
(i) alkanoic acids	8.0
(ii) alkandioic acids	21.1
Aromatic acid	1.5
Amino acid	0.7
Unidentified	7.9

TABLE-2
RELATIVE PERCENTAGE AND IMPORTANT MASS SPECTRAL PEAKS FOR METHYL ESTERS OF MONOMERIC COMPONENTS PRESENT IN DEPOLYMERIZED FRUITS CUTIN OF *C. SATIVUS*

Components	Relative percentage	Important peaks (m/z)
Alkanoic acids:		
Dodecanoic*	0.4	
Tetradecanoic*	1.4	
Pentadecanoic*	0.6	
Hexadecanoic*	9.9	
Hexadecenoic*	0.4	
Octadecanoic*	4.4	
Octadecenoic*	2.0	
Octadecandienoic*	2.1	
Octadecantrienoic*	2.3	
Eicosanoic*	0.3	
Docosanoic*	0.8	
Tetracosanoic*	0.3	
Hexacosanoic*	0.2	
Octacosanoic*	0.2	
Triacontanoic*	0.3	
Hexatriacontanoic	0.3	490[$M^+ - 60$]
Alkandioic acids:		
Nonandioic*	1.1	
Hexadecandioic*	2.2	
Octadecandioic	13.1	237[$M^+ - 105$], 236[$M^+ - 106$], 213, 199, 185, 171, 157, 143, 129, 115, 101
Octadecendioic#	10.3	234[$M^+ - 106$]
Docosandioic	3.3	398[M^+], 367[M^+—OCH_3], 280[M^+—OCH_3—$CH_2CH_2COOCH_3$]

Components	Relative percentage	Important peaks (m/z)
Hydroxyalkanoic acids:		
6-Hydroxytetradecenoic#	trace	256[M^+], 225[$M^+ - 31$], 143[α-cleavage], 125, 111
9-Hydroxytetradecantrieneoic#	trace	252[M^+], 234[$M^+ - 18$], 185, 97[both α-cleavage]
8,14-Dihydroxytetradecenoic#	0.6	254[$M^+ - 18$], 223[$M^+ - 18 - 31$], 222[$M^+ - 18 - (31 + H)$], 173[α-cleavage], 155[173 – 18], 141[100%, 173 – (31 + H)], 129[α-cleavage]
15-Hydroxyhexadecandienoic#	1.5	281[$M^+ - 1$], 267[α-cleavage], 249[$M^+ - 1 - (31 + H)$] and [267 – 18], 235[267 – (31 + H)]
8,9-Dihydroxyhexadecanoic		173[α-cleavage], 171[203 – (31 + H)], 155[173 – 18], 144[203 – 59], 141[173 – (31 + H)], 129[α-cleavage], 111[129 – 18]
8-Hydroxyheptadecanoic	~1.0	250[$M^+ - (31 + H) - 18$], 241[$M^+ - 59$], 225[$M^+ - 75$], 173, 157 [both α-cleavage], 141[173 – (31 + H)], 139[157 – 18]
8,9,18-Trihydroxyoctadecenoic#	0.8	201[α-cleavage], 185[203 – 18], 183[201 – 18], 173, 171[both α-cleavage], 153[171 – 18], 141[173 – (31 + H)], 135[171 – 18 – 18], 123[141 – 18]
13,14,18-Trihydroxyoctadecandienoic#		292[$M^+ - (31 + H) - 18$], 265[$M^+ - 59 - 18$], 264[$M^+ - (59 + H) - 18$], 251[269 – 18], 239[α-cleavage], 237[269 – (31 + H)], 207[239 – (31 + H)], 180[239 – (31 + H) – 18], 133[α-cleavage]
9,10,13,18-Tetrahydroxyoctadecanoic	1.4	275[α-cleavage], 253[$M^+ - (59 + H) - 18 - 31$], 235[253 – 18], 207[275 – (31 + H) – 18 – 18], 187, 175, 117 [all from α-cleavage]
8,9,14,18-Tetrahydroxyoctadecanoic		289[α-cleavage], 221[289 – (31 + H) – 18 – 18], 173[α-cleavage], 155[173 – 18], 141[173 – (31 + H)]

Components	Relative percentage	Important peaks (m/z)
8,12,13,18-Tetrahydroxyoctadecanoic	2.0	275[α-cleavage], 257[275 – 18], 245[α-cleavage], 213[245 – (31 + H)], 195[213 – 18], 173[α-cleavage], 155[173 – 18], 147[α-cleavage], 141[173 – (31 + H)], 117[α-cleavage]
16-Hydroxyeicosandienoic#	0.4	320[M^+ – 18], 281[α-cleavage], 249[281 – (31 + H)], 87[α-cleavage]
8,23-Dihydroxytricosanoic	0.3	257[α-cleavage], 239[257 – 18], 173[α-cleavage], 155[173 – 18], 141[173 – (31 + H)]
ω-Hydroxyalkanoic acids:		
18-Hydroxyoctadecenoic#	4.6	262[M^+ – (31 + H) – 18], 252[M^+ – (59 + H)], 234[252 – 18],
18-Hydroxyoctadecandienoic#		310[M^+], 278[M^+ – (31 + H)], 235[M^+ – 75]
Hydroxyalkandioic acids:		
6-Hydroxydodecan 1,12-dioic	0.9	225[M^+ – 31 – 18], 159, 145[both α-cleavage], 141[159 – 18], 127[159 – (31 + H)] and [145 – 18], 113[145 – (31 + H)]
2,11-Dihydroxydodecan 3,9-diene-1,12-dioic		286[M^+], 268[M^+ – 18], 254[M^+ – (31 + H)], 227[α-cleavage from both side], 227[M^+ – 59], 90[Mclafferty rearrangement]
7-Hydroxyhexadecan 1,16-dioic	1.6	201[α-cleavage], 169[201 – (31 + H)], 159[α-cleavage], 151[169 – 18], 127[159 – (31 + H)], 109[127 – 18]
8-Hydroxyhexadecan 1,16-dioic		187, 173[both α-cleavage], 155[187 – (31 + H)], 141[173 – (31 + H)], 137[155 – 18]
8-Hydroxyhexadecen 1,16-dioic#		269[M^+ – 59], 237[269 – (31 + H)], 187, 171[both α-cleavage]
8,10-Dihydroxyoctadecan 1,18-dioic	11.0	297[M^+ – 59 – 18], 187, 173[both α-cleavage], 155[187 – (31 + H)] and [173 – 18], 141[173 – (31 + H)] (100%)

Components	Relative percentage	Important peaks (m/z)
9-Hydroxyoctadecandien 1,18-dioic[#]	0.1	280[$M^+ - 74$], 199, 185[both α-cleavage]
5,10,11-Trihydroxyoctadecandien 1,18-dioic[#]	2.0	305[$M^+ - (31 + H) - 31 - 18$], 269[287 − 18], 251[287 − 18 − 18], 245[α-cleavage], 228[287 − 59], 201[α-cleavage], 197[215 − 18], 186[245 − 59], 171[α-cleavage], 139[171 − (31 + H)], 129[α-cleavage], 121[139 − 18]
8,10,11-Trihydroxyeicosan 1,20-dioic	4.6	275, 201, 173[all from α-cleavage], 169[201 − (31 + H)]
8,11,12-Trihydroxyeicosan 1,20-dioic		275, 261[both α-cleavage], 229[261 − (31 + H)], 213[231 − 18], 199[231 − (31 + H)] and [217 − 18], 187[α-cleavage], 181[199 − 18], 173[α-cleavage], 155[187 − (31 + H)], 141[173 − (31 + H)], 137[155 − 18], 123[141 − 18]
9,12,13-Trihydroxydocasan 1,22-dioic	0.5	289, 275, 245, 231, 201, 187[all form α-cleavage], 181[231 − (31 + H) − 18], 163[181 − 18], 155[173 − 18]
8-Hydroxydotriacontan 1,32-dioic	0.4	411, 173[both α-cleavage], 141[173 − (31 + H)], 123[141 − 18]
9-Hydroxydotriacontan 1,32-dioic		397, 187 [both α-cleavage], 155[187 − (31 + H), 137[155 − 18]
10-Hydroxydotriacontan 1,32-dioic		383, 201[both α-cleavage], 169[201 − (31 + H)], 151[169 − 18]
Aromatic acid:		
Benzoic*	1.5	
Amino acid:		
Hippuric*	0.7	

*Characterized from computerized library search facility.

[#] Position of double bond(s) undertermined.

appear to be the conventional method for GC-MS analysis, in the present study we have taken advantage of SP-1000 and SP-2100 SCOT capillary columns in analysing the cutin monomers as their methyl esters only. The efficiency of these columns in separating unsaturated (mono-, di- and tri-), saturated monobasic acids (even upto C_{36} acids) as well as their mono-, di-, and tri- hydroxylated derivatives and dibasic acids, more than compensated the relatively longer time required for gas chromatographic run.

The percentage distribution of monomeric components derived from depolymerization of *C. sativus* cutin are presented in Table-2. Some of the important mass spectral peak positions have also been incorporated in Table-2. Amongst the dibasic acids $C_{18}(C_{18:0}$ and $C_{18:1})$ constitute the major component (*ca.* 23%) while both C_{16} monobasic acids (10.3%, $C_{16:0}$ and $C_{16:1}$) and C_{18} monobasic acids (10.8%, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$) account for the major share (*ca.* 90%) of monobasic acids. The very long chain acids, C_{20}—C_{36}, constitute only 2.6% of the total monomers. Among the ω-hydroxy acids having no mid-chain hydroxylation, the unsaturated C_{18} acids ($C_{18:1}$ and $C_{18:2}$) are the dominant component (4.6%). So far as mid-chain hydroxy acids are concerned, the mono and dibasic acids having mono-, di- and tri- hydroxylation pattern have been found to occur. The presence of epoxy-hydroxy octadecanoic acid in *C. sativus* has already been reported[7]. Here also the C_{18} mid-chain hydroxy acids constitute about 60.0% of the total class whereas C_{16} acids represent only 11.0%. Some rare and minor components include 6-hydroxytetradecanoic acid, 8,14-dihydroxytetradecanoic acid, 13,14,18-trihydroxyoctadecanoic acid and 5,10,11-trihydroxyoctadecanoic acid. the presence of 8,10-dihydroxyoctadecanoic acid (11%) and octadecandioic acid (*ca.* 13%) from a cutin sample of *C. sativus* is really very interesting. The most outstanding feature of the results (Table-1) is that the composition of monomer as revealed in the present study is not exactly befitting with the classification of this phytopolymer as cutin or suberin. However, the designation of these microscopic anatomical entities based on chemical composition may not always be rationalised on the basis of the content of mid-chain modified monomers because some suberins might have substantial amount of these monomers. Probably the term "cutin-suberin like" or "mixed cutin-suberin" may be coined for such types of phytopolymers[8] having chemical characteristics in-between that of the cutin and suberin.

Benzoic acid (1.5%) is the only aromatic acid found in the present study while some minor phenolic acids are usual constituent of plant cutins. The identification of an amino acid, hippuric acid from *C. sativus* and *Cucurbita maxima*[9] is really very interesting because the occurrence of such acid probably represent its characterisation for the first time from any cutin sample.

The larger proportion of C_{18} family of monomers (53.4%) in comparison to members of C_{16} family (15.6%) in *C. sativus* fruit peel cutin is in line with the characteristic feature of slower growing fruits with well developed cuticle.

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